Cell Stem Cell
Previews

Rehabilitation of a Contract Killer: Caspase-3 Directs Stem Cell Differentiation

Mohammad Abdul-Ghani[1,2] and Lynn A. Megeney[1,2,*]
[1]The Sprott Center for Stem Cell Research, Regenerative Medicine Program, Ottawa Health Research Institute, 501 Smyth Road, Ottawa, ON K1H 8L6, Canada
[2]Department of Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON K1H 8M5, Canada
*Correspondence: lmegeney@ohri.ca
DOI 10.1016/j.stem.2008.05.013

Activation of caspase-3 is generally acknowledged as a penultimate step in apoptotic cell death pathways. Two studies in this issue of *Cell Stem Cell* (Fujita et al., 2008; Janzen et al., 2008) provide compelling data to demonstrate that caspase-3 is also a conserved inductive cue for stem cell differentiation.

Tissue development and maintenance are dependent on a complex interplay of stem cell self-renewal, differentiation, and apoptosis/programmed cell death. The phenotypic difference between renewal, differentiation, and death implies that each event is governed by a unique cohort of factors. In this issue of *Cell Stem Cell*, Fujita et al. (2008) and Janzen et al. (2008) report that the key apoptotic protease, caspase-3, mediates the differentiation of embryonic stem cells (ESCs) and hematopoietic stem cells (HSCs), respectively.

Based on the premise that stem cell differentiation corresponds with a loss of self-renewal capacity, Fujita et al. (2008) and Janzen et al. (2008) conducted a series of elegant studies to explore the role of caspase-3 as a probable gatekeeper of stem cell function. Using an inducible caspase-3-specific cleavage sensor, Fujita and colleagues observed that initiation of caspase activity coincided with early stages of ESC differentiation in otherwise healthy cells. Further, caspase-3 null ESCs displayed significant defects in in vitro assays of differentiation as well as an inability to form differentiated progeny when transplanted in vivo. The authors reasoned that caspase-3 mediated its effects through targeted cleavage of a pluripotent factor(s), demonstrating Nanog to be a priority target/substrate. Consistent with this hypothesis, expression of a caspase-3-resistant Nanog promoted ESC self-renewal while inhibiting differentiation. The results of Janzen et al. (2008) were equally persuasive in linking caspase-3 to the differentiation of HSC. Using a null mouse model, the authors established that loss of caspase-3 resulted in the accumulation of phenotypically defined long-term (LT) repopulating HSCs, with a corresponding reduction in circulating mature hematopoietic cells. In addition, no changes in the percent of apoptotic HSCs were observed. The observed differentiation defect was shown to be cell autonomous, as transplanted bone marrow from caspase-3 null animals replicated the aberrant hematopoietic lineage profiles in wild-type recipient mice. Interestingly, cytokine responsiveness and cytokine-mediated signals were elevated in caspase-3 null HSC, yet this alteration did not appear to be dependent on the enzymatic activity of the protease. Together, these findings provide compelling evidence that a protein with an established apoptotic role is also indispensable for the regulation of stem cell development and differentiation.

The discovery of a nondeath role for caspase-3 in stem cell self-renewal and differentiation appears to be counterintuitive. For example, stem cells and their immediate progeny need to remain free of factors that can cause lasting cell damage if they are to maintain pluripotency, genome integrity, and LT survival. Typically, the activation of the proteolytic enzyme caspase-3 acts as a convergent point for a number of death-signaling pathways. Once engaged, the effecter caspase enzymes, such as caspase-3, -6, and -7, cleave vital protein substrates, an event that precipitates many of the morphologic changes associated with cell death (Fischer et al., 2003). Given the wealth of observations that have positioned caspase-3 as the arbiter of the death signal, how is it then possible that this enzyme could do anything other than kill a stem cell?

The answer to this question may arise with a deeper appreciation of the mechanics that govern cell-fate decisions. Indeed, while there exists an obvious contradiction in the final outcomes of apoptosis and differentiation, there is also a remarkable degree of morphologic and biochemical symmetry between each process. Cells undergoing either apoptosis or differentiation display comparable cytoskeletal rearrangements, membrane fusion, and fission events as well as similar alterations in chromatin and nuclear architecture (Fernando and Megeney, 2007). This parallel is most evident in cells that engage a differentiation phenotype resembling attenuated cell death (i.e., are enucleated and short lived) such as red blood cells and epithelial derivatives. The differentiation program in these cell lineages has been shown to be dependent on a transient caspase-3 signal, unlike the sustained caspase-activation profile that triggers apoptosis (Ishizaki et al., 1998; Zermati et al., 2001). Similarly, transient activation of caspase-3 also mediates differentiation of longer-lived cell types such as skeletal muscle, osteoblasts, and neurons (Fernando et al., 2002; Miura et al., 2004; Fernando and Megeney, 2007). The observations from the current studies greatly extend this paradigm, suggesting that caspase-3-directed differentiation is a primordial feature of the cell-fate decision-making process (Figure 1A).

The primary interpretation of Fujita et al. (2008) and Janzen et al. (2008) is that caspase-3 promotes stem cell differentiation indirectly, by limiting self-renewal per se rather than directly engaging a differentiation program (Figure 1B). A corollary to

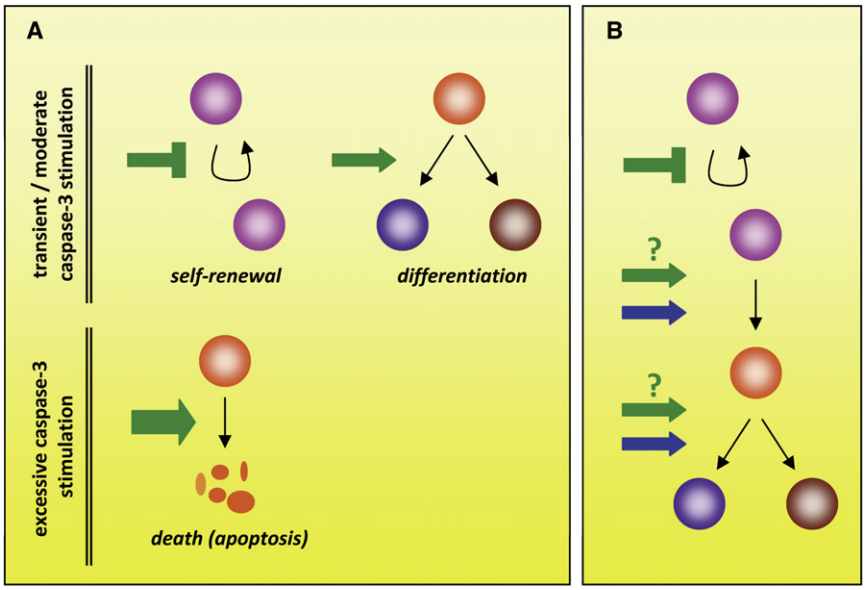

Figure 1. Caspase Regulation of Cell-Fate Decisions
(A) Caspase activation (green arrows) induces stem cell self-renewal, cell differentiation, or cell death (apoptosis). The specific outcome may depend on the extent and/or kinetics of caspase activation.
(B) Fujita et al. (2008) and Janzen et al. (2008) postulate that caspase-3 indirectly promotes stem cell differentiation by limiting self-renewal of the stem cell pool, hence favoring differentiation. Other, largely unidentified, factors (depicted with blue arrows) are also likely to participate. Alternatively, caspase-3 may influence differentiation more directly, by actively engaging factors that promote the gene expression and phenotype of a differentiated cell type, in addition to limiting self-renewal.

this hypothesis is that additional factors (as-of-yet undefined) promote the stem cell differentiation program following caspase blockade of self-renewal. Data from both studies demonstrate that caspase inhibition leads to a dramatic increase in the stem cell population, and Fujita and colleagues have shown that caspase-3 limits ESC pluripotency through direct cleavage of one protein alone, Nanog. An alternative interpretation of the current results may also be proposed. That is, caspase-3 may participate in stem cell differentiation in a more direct fashion rather than by simply limiting self-renewal. In this model, caspase-3 may simultaneously engage factors to promote the gene expression profile and resulting phenotypic changes that result in a specific differentiated cell type (Figure 1B). In potential support of this hypothesis, Janzen and colleagues have shown that loss of caspase-3 results in a dramatic reduction in early B lymphocyte maturation, a step far removed from the self-renewal process of an HSC. This multitask interpretation is also more consistent with previous observations that have demonstrated a direct role for caspase-3 in the terminal differentiation of numerous lineage-committed cell types (as noted above). A definitive position for either model will require a more complete mapping of potential caspase substrates.

Collectively, the work of Fujita et al. (2008) and Janzen et al. (2008) suggests that caspase-3 acts at multiple steps in the stem cell life cycle, affecting both self-renewal and differentiation. Yet, like all exciting observations, the solution to one mystery raises so many more questions. Paramount among these, how is caspase-3 activity restrained and guided to influence stem cell differentiation rather than cell death? The study of cell differentiation in other systems may provide the answer. *Drosophila* spermatid differentiation is dependent on the entire apoptotic signal cascade that culminates in activation of caspase homologs (Huh et al., 2004). However, caspase activity in this system is tightly scripted through ubiquitin-mediated degradation of caspase-inhibitory proteins (Arama et al., 2007). Such a mechanism ensures a level of caspase activity that is sufficient to drive spermatid differentiation yet not high enough to engage an apoptotic program. Whether this or other control mechanisms operate in various mammalian stem cells remains unknown.

REFERENCES

Arama, E., Bader, M., Rieckhof, G.E., and Steller, H. (2007). PLoS Biol. *5*, e251. 10.1371/journal.pbio.0050251.

Fernando, P., and Megeney, L.A. (2007). FASEB J. *21*, 8–17.

Fernando, P., Kelly, J.F., Balazsi, K., Slack, R.S., and Megeney, L.A. (2002). Proc. Natl. Acad. Sci. USA *99*, 11025–11030.

Fischer, U., Janicke, R.U., and Schulze-Osthoff, K. (2003). Cell Death Differ. *10*, 76–100.

Fujita, J., Crane, A.M., Souza, M.K., Dejosez, M., Kyba, M., Flavell, R.A., Thomson, J.A., and Zwaka, T.P. (2008). Cell Stem Cell *2*, this issue, 595–601.

Huh, J.R., Vernooy, S.Y., Yu, H., Yan, N., Shi, Y., Guo, M., and Hay, B.A. (2004). PLoS Biol. *2*, e15. 10.1371/journal.pbio.0020015.

Ishizaki, Y., Jacobsen, M.D., and Raff, M.C. (1998). J. Cell Biol. *140*, 153–158.

Janzen, V., Fleming, H.E., Riedt, T., Karlsson, G., Riese, M.J., Lo Celso, C., Reynolds, G., Milne, C.D., Paige, C.J., Karlsson, S., et al. (2008). Cell Stem Cell *2*, this issue, 584–594.

Miura, M., Chen, X.D., Allen, M.R., Bi, Y., Gronthos, S., Seo, B.M., Lakhani, S., Flavell, R.A., Feng, X.H., Robey, P.G., et al. (2004). J. Clin. Invest. *114*, 1704–1713.

Zermati, Y., Garrido, C., Amsellem, S., Fishelson, S., Bouscary, D., Valensi, F., Varet, B., Solary, E., and Hermine, O. (2001). J. Exp. Med. *193*, 247–254.